11. Pharmacokinetics and Toxicology

11.001 Toxic effects of OMC administration during development of rats in lactational period. Barbosa E, Savignon T, Ferraris FK, Chaves AS, Muylaert FF, Rodrigues SA, Brito TM, Amendoeira FC Fiocruz – Farmacologia e Toxicologia

Introduction: UV radiation might lead to several deleterious effects to human health, among them DNA damage, early aging, cataract, chemical unbalance e skin lesions, immunosuppression, melanome. Because these effects there is the need to protect the population against UV radiation, by using cosmetics and other products that act as UV filters. The consuming market presents a series of these filters, which are classified as organic or inorganic ones. The octyl methoxycinnamate (OMC), an organic UV filter, is widely used in cosmetics, sunscreen, shampoos, among others, all of them registered in Brazil. Several studies have been shown that OMC is present in biological fluids, as blood, urine, milk, water as well as in chain food. The safety of use of these products is a great concern, once several studies have shown that OMC is a potential endocrine disruptive, especially on thyroid axis. Thyroid hormones are crucial during the development, the Central Nervous System one of the systems most affected by any disturbances in thyroid axis. Thyroid hormones have important role in proliferation, neuronal migration and maturation, and have been implicated alterations in several systems such cardiovascular system and development endpoints. **Objective**: This present work aims to evaluate if a thyroid disruption induced by OMC administration in rats during lactacional period causes developmental alterations. **Methods**: All experiments receive approval from our ethical committee, number P-16/14.2. Pups were sexed and divided in two exposition groups in post-natal day 1 (PND), corn oil or OMC in three different doses (5, 50 or 500 mg/Kg/dose). The rodents received daily one dose of OMC from PN5 to PN22. In this period, development parameters were observed such as hair growth, tooth eruption and eyes open. In PN23, day of weaning, the animals were euthanized to collection of blood for total T4 dosage and organs such as pituitary, hypothalamus, thyroid, liver, ventricles and brown-fat tissue for further analyses. **Results**: The OMC exposition statistically decreased the weight gain of animals from both sexes of group 500 mg/Kg/day compared to corn oil group. The mean difference of OMC in larger dose compared to corn oil group was 3,486 ± 0,8284 (p value <0,001). A decreased total T4 was observed in OMC same dose in PN23, when compared to corn oil group (% of control), at both sexes: OMC 85.2% ± 2.6% (ANOVAr; df = 3; F = 7,175; p=0,002). Regarding eye opening, chi-square analyses showed a mild delayed in OMC 500 mg/Kg/day group compared to corn oil (X^2= 2.5; p< 0.1).<p).<p).</p).<p).Hair growth and tooth eruption displayed no significance differences on other tested doses. The biochemical analyses, such as creatinine, uric acid, albumin, cholesterol and total proteins presented no differences. **Conclusion**: Our results shown that OMC exposition statistically decreases gain weight during lactation suggesting a toxicity signal. Furthermore, decreased total T4 indicate a compromised thyroid axis development by OMC. A neurodevelopment milestone, eye opening, was mild delayed in OMC 500 mg/Kg/day group. More studies are necessary for to clear the action mechanism of OMC. **Financial Support**: Fiocruz,PAPES/CNPq.

11.002 Determination of free tissue brain concentration of voriconazole by microdialysis in healthy and cryptococcus neoformans infected Wistar rats.
Alves IA[1], Lock G[2], Rist J[2], Rates S[1], Araújo BV[1] [1]UFRGS – Ciências Farmacêuticas, [2]UFRGS – Farmácia

Introduction: Cryptococcosis is a systemic infection caused by Cryptococcus spp that often is associated with meningitis. Among the species, *Cryptococcus neoformans* stands out for the high mortality (70-90%), and is directly related to infections in immunocompromised and HIV patients. Fluconazole and amphotericin B are the drugs commonly used to treat cryptococcal meningitis, thus, voriconazole (VRC) is an alternative to pharmacotherapy, when the strains become resistant to fluconazole, being less toxic than amphotericin B. Considering the site of infection, it is important to know the free fraction of drug that is able to access the brain, once this responsible by the pharmacological response. It is known that tissue penetration depends on variables such as diffusion, active transport, lipid solubility and protein binding; CNS infections, some may promote penetration of drugs. Therefore, cerebral microdialysis appears as a technique for determining the continuous brain concentrations of drugs through an in vivo assay. **Aims**: In this context, given the lack of data considering the free VRC fraction achieved in brain, this study aims to evaluate free levels of VRC in brain of healthy and *C. neoformans* infected Wistar rats employing microdialysis, and to establish the relationship between free brain and free plasma levels in both conditions. **Methods**: Animals were infected by 100µL injecting $1 \cdot 10^5$ CFU inoculum trough the tail vein (n= 6/group). After 8 days, they were submitted to surgery for to insertion a microdialysis probe (CMA® 12) was inserted into the brain tissue in the region of the primary motor cortex of animals (1mm x 2,0mm x 3.2mm) [1]. After probe implantation was allowed 48 hours animals recovered (protocol approval by the Animal Ethics Committees at UFRGS # 26605). In both groups (healthy and infected) 5 mg/kg of the VRC was administered i.v.. Blood and microdialysate samples were collected at pre-determined time points up to 8h. Total plasma and free tissue concentrations ($C_{tissue,free}$) were determined by LC-MS/MS and LC-UV, respectively. **Results**: Total plasma AUC_{0-t} of data from healthy and infected animals were 20.74 and 27.26 µg/ml, respectively. The tissue data showed a high penetration of difference between the healthy and infected animals with average ratio of free tissue/free plasma (fT) of 1.07 for healthy and 2.14 for infected. The results showed excellent penetration into the tissue of VRC and their potential for the treatment of fungal meningitis, because the AUC_{0-t} of the free tissue was two times higher than the levels found in plasma. **Conclusion**: Pharmacokinetic results indicate that a meningococcal infection caused by *C. neoformans*, increases the brain penetration of VRC, indicating excellent tissue penetration of this antifungal drug in the infected tissue and its potential for the treatment of fungal meningitis. The plasma data showed differences between groups (healthy and infected), emphasizing the importance of tissue determinations of drugs, local actuation thereof and where they should be at concentrations appropriate to ensure treatment efficacy. **Acknowledgements**: Financial support from CNPq. **References**: 1.Paxinos & Watson, 3rd Ed, Academic Press, 1997.

11.003 Evaluation of potential toxicity of hydroethanolic extract of *Terminalia argentea* **Mart Leaves.** Beserra AMSS, Martins DTO UFMT – Ciências Básicas em Saúde

Introduction: The infusion prepared from leaves of *Terminalia argentea* (Combretaceae) is used in Brazilian popular medicine in the treatment of wound, gastric ulcers and inflammation. **Aims**: To evaluate cytotoxicity, acute and subchronic toxicity of the hydroethanolic extract of T. argentea (HETa), in *in vitro* and in vivo experimental models. **Methods**: In Hippocratic screening, adult Swiss mice (n=6/group), of both sexes, received by gavage vehicle or HETa (1000 or 2000mg/kg), and were observed individually for a period of 8h on the first day and, once a day, for 14 days, and clinical signs and symptoms were noted in a Table adapted from Malone. Finally, the weight gain (%) at 14 days was determined and the vital organs were weighed and necropsied. The cytotoxicity of the HETa was evaluated by Alamar Blue method in CHO-k1 and AGS cell lines and determining IC50 at 24 and 72h. Subchronic toxicity was evaluated by single daily oral exposure of Wistar rats to the vehicle or HETa (50, 200 and 800mg/kg), for 30 days. The animals were kept in individual metabolic cages and behavioral changes, water and food consumption, excretion of feces and urine were evaluated. At the end of the period, blood was collected for determination of haematological and biochemical parameters, while some vital organs were harvested for autopsy and determination of relative weights. **Results**: In Hippocratic test, there was piloerection, passiveness and loss of seizure paw in male mice, these effects were non dose-dependent and reversible at 2h. The same effects as noted in the male mice were observed in the females, all were reversible within 4 days. In addition, tail erection and diarrhea, were reversible at 8h and 6 days, respectively. HETa doses up to 2000mg/kg caused no lethality or alterations in body weight and increase the relative weight of the vital organs of the mice, except in the stomach relative weight of male mice treated with 1000mg/kg, which increased by 21.6 % ($p<0.05$) compared to vehicle (0.72±0.05). In the cytotoxicity assay, HETa presented IC50>200µg/mL in CHO-k1 and IC50>400 µg/mL in AGS, at times 24 and 72h. In subchronic toxicity, HETa caused no behavioral changes nor in the accumulated weight gain, water consumption, excretion of feces and urine in rats. However, at 800mg/kg there was a reduction (18.6%; $p<0.01$) in food consumption in the 19th day compared to the vehicle (37.6±0.72g). This dose reduced relative weight of heart by 9.8% ($p<0.05$) relative to the vehicle (0.01±0.36). Biochemical parameters were not changed except at HETa dose of 50mg/kg which reduced the levels of GPT ($p<0.05$) by 5.2% relative to the vehicle (65±2.7 IU/L). The total leukocyte counts increased by 6.9% ($p<0.01$) and the number of neutrophils by 37% ($p<0.001$) in the 200mg/kg group compared to the vehicle (6.6±0.24x10^3/µL and 1.2±0.11x10^3/µL, respectively). The dose of 800mg/kg caused increase of 194.8% ($p<0.01$) in the number of monocytes in relation to the vehicle (0.016 ± 0,003x10^3/µL). The lungs of a rat treated with 200mg/kg and two with 800mg/kg EHTa presented more blackened and hemorrhagic spots. **Conclusion**: HETa has low acute and subchronic oral toxicity, requiring findings of histopathological analysis to confirm or not the signs described in this study. Financial Support: FAPEMAT; CAPES/Pró-Amazônia. Animal Research Ethical Commitee: CEUA/UFMT n^{o}23108.030953/14-7.

11.004 Determination of thimerosal content in Influenza A (H1N1) multi-dose vaccine and evaluation of *in vitro* toxicity. Rodrigues S[1], Ferraris FK[1], Leandro KC[2]
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Introduction: Thimerosal (sodium ethylmercuri-thiosalicylate) is a water-soluble derivative of thiosalicylic acid with antimicrobial activity. Thimerosal is used as an antimicrobial agent and a preservative in cleaning solutions for eye lenses, cosmetics, and widely used in vaccines. The safety of thimerosal has recently been questioned based on a number of studies that indicate to its possible risk of toxicity. During the last years there has been major concern about the possible risk of thimerosal exposure to children through routine immunization. It is estimated that an infant could be exposed to a high concentration of thimerosal from the routine immunization schedule. U.S. Food and Drug Administration (FDA) has published their recommendations to remove thimerosal from vaccines routinely given to infants. In Brazil, Influenza A (H1N1) multi-dose vaccine contains thimerosal, but it has not yet been established an acceptable limit concentration for these preservative. In diphtheria, tetanus, meningitis and rubella vaccines, the Brazilian Official Pharmacopoeia recommends a limit of 200 ppm (500 µM) of thimerosal. **Aim**: To determine the thimerosal content on H1N1 vaccines distributed in Brazil and to evaluate *in vitro* toxicity of thimerosal in THP-1 human macrophage cell line. **Methods**: Thimerosal quantification was determined by Hanging Mercury Drop Electrode (HMDE), using a polarography system (Metrohm 694 VA Stand). Cytotoxicity of thimerosal in THP-1 cell line (human macrophage cell line) was assessed using an alamarBlue™ Reduction assay. Viable cells were seeded in a flat-bottomed 96-well plate and incubated in the presence of different concentrations of thimerosal (0.1, 0.5, 1, 5, 10, 50, 100 and 500 µM) for 48 h. After a period of 48 h, cell viability was measured by the resazurin metabolism assay, in which resazurin reduction to resorufin by living cells can be assessed by fluorescence using a microplate reader at wavelengths of 570 and 600 nm (SpectraMax M5). For apoptosis analysis, THP-1 cells were incubated with annexin V - 7 AAD apoptosis kit for 20 minutes. After the incubation period, samples were analysed by flow cytometer (CyFlow Space). **Results**: A set of sixteen H1N1 vaccines was tested. Was determined an average concentration of thimerosal from 40 to 48 ppm, equivalent to 100 to 110 µM. Based on this results, THP-1 were exposed to different concentrations of thimerosal (0.5, 1, 5, 10, 50, 100 and 500 µM) for 48 h. Thimerosal's concentration of 10 to 500 µM were toxic, leading to cell viability values below 10% (all doses used above 10 µM of thimerosal induced 90% of cell death). To determine the mechanism involved in this low viability, we evaluated thimerosal-induced apoptosis. Thimerosal (5 to 500 µM) induced cell apoptosis markers (annexin $V^+/7AAD^-$ and/or annexin $V^+/7AAD^+$) after 3, 6 and 24 hours of incubation. **Conclusions**: Concentrations of thimerosal in the H1N1 vaccine samples are within the limits established by the Brazilian Official Pharmacopoeia ($\leq$ 200 ppm), however these concentrations show severe toxicity in human macrophage cells, leading to apoptosis after 3h of contact. **Financial support** FIOCRUZ.

11.005 Development a diabetic model with streptozotocin in Wistar rats applied to a microdialysis study. Izolan JS, Braga A, Lima DMF, Araújo BV UFRGS

Introduction: Diabetes mellitus is a complex metabolic disease affecting around 5% of people all over the world. New drugs are continually being tested and new strategies developed to prevent and treat diabetes. Experimental animal models of diabetes have been used in this purpose. Streptozotocin (STZ) and Nicotinamide (NA) are often associated in a model used to induce diabetes in rats [1]. Streptozotocin cause pancreatic □-cell damage, which are the only source of insulin, while nicotinamide partially protect insulin- secreting cells against STZ. The partial protection exerted by nicotinamide against the β-cytotoxic effect of STZ results in an experimental diabetic syndrome in rats that appears closer to diabetes insulin non dependent. [2] The objective of this study was induce an experimental diabetes mellitus by streptozotocin and nicotinamine in Wistar rats and evaluate levels reached in liver of diabetics animals by metformin, using microdiaysis. **Method**: Male Wistar rats (n=6) weighting 190-330g received intraperitoneal administration of NA (100 mg/kg) dissolved in saline 15 min before an intravenous administration of 65 mg/kg STZ in citrate buffer (pH 4.5) to overnight fasted Wistar rats. Twenty four hours and 3 weeks after the induction, animal caudal blood was collect and blood glucose was measure using a glucose meter (Accu-check Performa®). Pharmacokinetic study was carry out with anesthetize rats using ethyl carbamate (1.25 g/kg, i.p.) after that the microdialysis probes (CMA 20) were insert into the liver. After i.v. administration of metformin (50 mg/kg) the dialysates were collected each 30 min up to 12 hours. All experiments were approve by Committee of Ethics in Animal Use - UFRGS (25780). The non-compartmental analyses were done using Excel®. **Results**: Animals showed basal glucose - one day before the induction - of 486.5 ±34.8 mg/dL, twenty four hours after STZ and NA induction the blood glucose of all animals was higher than 250 mg/dL, 3 weeks after the induction the blood glucose was 486.5 ± 34.8 mg/dL, and all animals had satisfactory weight gain, characterizing the diabetic scenario. Pharmacokinetic study of metformin liver showed lambda 0.27 ± 0.25 h^{-1}, half-life 4.85 ± 4.43 h^{-1}, $AUC_{0\text{-}inf}$ 175.24 ± 98.94 µg*h/mL. **Conclusion**: The model showed be able to induce the diabetes mellitus in Wistar rats characterized by higher blood glucose (> 250 mg/dL), allowing apply it to disease pharmacokinetic study. **Acknowledgements**: Financial support from CAPES. Committee of Ethics in Animal Use - UFRGS (25780). **References**: 1. Szkudelski, Experimental Biology and Medicine, v. 237, p. 481-491, 2012. 2. Masielo et al. Diabetes, v. 47, p. 224, 1998.

11.006 Accessing metformin free levels in healthy and diabetics rat tissues using microdialysis technique. Braga A[1], Izolan JS[1], Lock GA[2], Dalla Costa T[1,2], Araújo BV[1,2] [1]UFRGS – Ciências Farmacêuticas, [2]UFRGS – Faculdade de Farmácia

Introduction: Pharmacokinetic studies and clinical therapeutic monitoring of most drugs usually use the plasma concentration, without considered the free drug concentration, which are responsible for the pharmacological action. Furthermore, the measured of plasma drug concentration does not always reproduce the free fraction on target tissues. Microdialysis technique allow the measure of pharmacological drug fraction into the plasma or target tissue [1]. Metformin is the drug most recommended as first-line treatment by the international diabetics guidelines [2]. Metformin plasmatic concentration has been described in the literature however, no study of drug's unbound fraction achieved in the target tissue has been reported. This study aims to determine the free tissue concentration of metformin in liver (target tissue) and muscle (feasible tissue to be evaluated in clinical scenarios) of rats by using microdialysis. **Methods**: The diabetes mellitus was induce in male Wistar rats weighted 180 – 330g according [3]. Animals received an intravenous administration of streptozotocin (65 mg/kg) 15 minutes after the nicotinamide (100 mg/kg; i.p.) administration. Glucose was measure one day before (basal) and one day after the diabetes induction, the animals that showed plasmatic glucose higher than 250 mg/dL were consider diabetic. Healthy and diabetics rats were anesthetize with ethyl carbamate (1.25 g/kg, i.p.) and microdialysis probes (CMA 20) were perfused with Ringer's solution and inserted into the liver and muscle. After i.v. administration of metformin (50 mg/kg) the dialysates (45 µL) were collected each 30 min up to 12 hours (at flow rate of 1.5µL/min). Metformin was quantified by previous HPLC validated method developed in our laboratory. All experiments were approve by Committee of Ethics in Animal Use - UFRGS (25780). The non-compartmental analyses was done using Excel® and all pharmacokinetics parameters were compared by Student's t test ($p < 0.05$) using the software SigmaStat®. **Results**: Liver and muscle pharmacokinetic parameters of metformin were the same when we compared the tissues in the same group – healthy (n=11) or diabetic (n=4) - $p>0.05$. Disease also has not changed pharmacokinetic parameters in both tissues when compared healthy vs diabetic animal ($p > 0.05$). Liver showed lambda 0.36 ± 0.20 vs 0.27 ± 0.25 h^{-1}, half-life 2.56 ± 1.42 vs 4.85 ± 4.43 h^{-1}, $AUC_{0\text{-inf}}$ 287.64 ± 358.25 vs 175.24 ± 98.94 µg*h/mL in healthy and diabetic animals. Muscle pharmacokinetic profile showed lambda 0.31 ± 0.12 vs 0.29 ± 0.24 h^{-1}, half-life 2.58 ± 1.16 vs 4.66 ± 4.36 h^{-1} and $AUC_{0\text{-inf}}$ 216.89 ± 138.49 vs 226.92 ±158.66 µg*h/mL in healthy and diabetic animals**.** Conclusions: Pharmacokinetic profile of liver and muscle are the same in healthy and diabetic animals. It allows inferring that the muscle leg concentration is the same than the liver. Besides that, the disease seems not change the metformin penetration into the tissues. **Acknowledgements**: Financial support from CNPq, FAPERGS. Committee of Ethics in Animal Use – UFRGS process number 25780. **References**: 1. Azeredo, F.J. et al. Clin Pharmacokineti, v.53, p. 205, 2014. 2. Choi, H. et al. J Pharm. Sci., v.95, p. 2543, 2006. 3. Masielo et al. Diabetes, v.47, p.224 1998.

11.007 A post-marketing study of pharmacokinetic bioequivalence between commercial generic and reference amoxicillin in rats. Mattos LIS, Ferraris FK, Brito TM, Chaves AS, Martins HF, Pinto DP, Silva DMD, Amendoeira FC. LAB-SEFAR-Fiocruz

Introduction: After almost 16 years since the beginning of the Brazilian generic medicines politics in 1999, the acceptance from physicians and the general population is unlike that in other countries that have adopted them for a longer time. The main distrust of consumers regarding the quality of the product lies on its lower cost and the association the word “generic” has with some expressions that infer something of poor quality or inefficient, which gives the product pejorative character. Even though the quality of the products is guaranteed by Resolution RDC No. 17/2010, which regulates good practice for the manufacturing of drugs, post-market quality control is still poor. The verification of the product, except for registration and renewal purposes, is currently only performed when there are reports or many cases of adverse events regarding a drug. In these cases, the tests made are content, organoleptic characteristics, microbiological and potency related. However, none of these tests yields results that prove the bioavailability of the drug. Thus, it is necessary to carry out studies to prove the safety and efficacy of generic drugs available in Brazilian market to promote a greater adherence to the program. **Objective**: The aim of this study was to develop an animal model to test the bioavailability / bioequivalence of generic drugs and perform an bioequivalence study to compare three different generic formulations marketed in Brasil. **Methods**: The animals were divided in 4 different groups of 6 animals each and fasted 12 h before oral administration (1 ml of 3 mg/kg suspension) of the innovator or one of 3 different brands of commercial amoxicillin suspension (500 mg) (The manufacturer names were suppressed to avoid legal disputes). The blood samples were collected in time 0, 10, 20, 40, 50, 60, 120, 180, 300, 420 and 540 min. after administration. At the end of the blood collection the animals were kept in the Laboratory Animal Facilities during the washout period (1 week), ensuring complete physical recovery of the animal, and after this period they were again tested with a different drug, following a cross-over design based on Williams design (all 24 animals were tested to the 4 different drugs). The plasma amoxicillin quantification was performed using a Pursuit C18 column 150.0 mm X 3.0 mm X 5.0 µ coupled to mass spectrometry, An ABSciex QTRAP 5500.This method showed good linearity (10, 25, 100, 500, 1000, 2000, 2500 ng ml-1), precision, accuracy and stability and showed be simple, fast and efficient in conducting this amoxicillin assay. **Results and Discussion**: The results of AUC obtained in this study shows one of the three test formulations failed to achieve the bioequivalence when compared to reference drug(*). The calculated AUC was 106353.48 ± 6452.37 to reference drug, and 97841.05 ± 8494.10*; 101793.22 ± 6747.73 and 110309.63 ± 7739.21for the three generics tested. Our results show the need to review quality control models currently being used. And gives another and important option for the quality control of medicines and also can be used in new drugs development and medicines. **Financial support**: APQ1/Faperj and PAPES/CNPq Ethical license (CEUA/FIOCRUZ; P49/13-3)

11.008 Histopathological evaluation of the profile of non-human primate species of *Cebus apella* treated with LDE-paclitaxel oleate as a tool for cancer therapeutics. Oliveira NCL[1], Feio DCA[1], Silva WB[2], Muniz JAPC[2], Burbano RR[1], Maranhão RC[3], Lima PDL[4] [1]UFPA, [2]CENP, [3]Metabolismo de Lípides, [4]UEPA

Introduction: In previous studies, develops a serving chemotherapeutic system, called LDE with lipid composition similar to that of the natural LDL, this has advantages over commercial chemical forms as it is able to concentrate in tumor tissue after injection into the current circulatory and can thus target tumors to drugs incorporated into the nanoemulsion. The LDES can be used as carriers of chemotherapy paclitaxel (PTX) for possible reduction of toxicity and increase their therapeutic action.In clinical studies evaluating the pathology profile (macro and microscopic) is extremely important because it analyzes the structure and function at the cellular level, allowing analyze the health of the body, monitor the progress of treatment and enable the evaluation of therapeutic response of new drugs. Objective: To evaluate the chronic toxicity of nanoparticles associated with paclitaxel chemotherapy (LDE-PTX) in individuals of Cebus apella species, from the determination of prognostic parameters, and possible changes.**Methods**: The project was approved by the ethics committee in research with animal models of UFPA (BIO008-11). 15 animals were used in Cebus apella species, divided into different groups: negative control (NC); Experimental (EXP1 and EXP2) where animals received LDE-PTX intravenously at two different doses of 175 mg / m² and 250mg / m² respectively; and the positive control (CP1 and CP2) animals received the drug intravenously in commercial form at the same doses used in the experimental group, respectively. The animals were observed for 6 cycles of chemotherapy with 3 week intervals. After treatment the animals were euthanized and the tests performed histopathological liver, spleen, and kidney.Results and **Discussion**: The toxicity in the liver was evidenced by the presence of Sinusoidal bleeding, micro and macrovesicular steatosis in the groups EXP 2 and CP 1 and 2. The group EXP 2 also showed focal necrosis of hepatocytes which may be linked to hepatic toxicity related to chemotherapy, in this same group was observed swelling, which can be characterized by intensive work of this organ for drug metabolism. The nephrotoxicity caused by paclitaxel and PTX-LDE shown by glomerular and tubular hemorrhage and accumulation of hyaline casts in the tubular lumen in the group EXP 2. Furthermore, the presence of inflammatory infiltrate us kidneys revealed that doses of groups CP1 and 2 and EXP 2 demonstrate evidence of toxicity renal. In the analysis of the spleen was observed that the groups EXP 2 and CP 1 and 2 are with splenomegaly,this modification may be connected the hepatic disorder. **Conclusion**: This study revealed a lower toxicity in the animals treated with PTX-LDE in the group EXP 1 in the lower dose, 175 mg/m2, featured by the absence of significant toxicity in histopathological analysis of liver in the animals mentioned.**Financial support**: CNPq, CAPES, CENP and FAPESP.

11.009 Evaluation of the effects of mangiferin nanocapsules on hematological parameters in wistar rats. Garcez RA, Carmo GM, Raffin R, Fontana BD, Borin DB, Vaucher RA, Rech VC Centro Universitário Franciscano

Mangifera indica L., known as mango, has a major compound, mangiferin (1, 3, 6, 7-tetrahydroxy-xanthone-C2-bd-glucosylated), which is a natural bioactive and can be obtained from leaves and fruits, as well as their cortex. The mangiferin has several medicinal properties including up quotes: antibacterial, antidiabetic, against rheumatoid arthritis, antipyretic activity, antidepressant and anxiolytic, anti-inflammatory, anti tumoral, antioxidant. However, the mangiferin has a low solubility in water. Thus, one strategy used to increase their solubility and hence their therapeutic efficacy is nanoencapsulation. This study aimed to verify the subchronic toxicity of the nanocapsules containing mangiferin (NCM) in Wistar rats on the hematological profile. The suspensions were prepared by adaptation of the technique of interfacial deposition of preformed adapted polymer. And the concentration of mangiferin was 0.25 mg/ml of suspension. After the production physico-chemical characterization of the suspensions of NCM was carried out, to assess toxicological profile was made a subchronic treatment of 24 days. This work was carried out after approval by the Ethics Committee on Animal Use from Centro Universitário Franciscano, under protocol number 003/2014. For subchronic treatment two ways of administration were used: oral and intraperitoneal. The animals were divided into 5 groups (n=8) for each way of administration described: Control Group (C), 0, 85% of saline solution was administered orally; Vehicle (V), where it was given orally 0.1 mg/kg of rats; Mangiferin group (M), where it was given orally 0.1 mg/kg of rats; Nanocapsules white group (WNCs), where it was given orally 0.1 mg/kg of rats; Nanocapsules of mangiferin (NCM), where it was given orally 0.1 mg/kg of rats. After the period of 24 days the animals were killed by decapitation, blood was collected for hematological analysis. Total leukocytes (WBC), total erythrocytes (RBC), hematocrit (Ht), hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and platelets were determined. Were obtained with particle sizes of 69.1 ± 8.1 nm, polydispersity index (0.12 ± 0.06) and the zeta potential was -32.35 ± 4.03 mV. It can be observed the effect of orally treatment on hematologic parameters, where was identified a significant increase of MCHC in the groups WNCs ($P<0.05$) and NCM ($P<0.001$) when compared to the control. And the effect of intraperitoneally treatment on hematologic parameters, it can be observed that a significant decrease of Hb ($P<0.01$) was identified, Ht and MCHC on group NCM. Also a significant increase of WBC ($P<0.001$) was identified, neutrophils and immature neutrophils in the groups WNCs and NCM when compared to the control. Based on these results we conclude that the polymeric Nanocapsules with mangiferin showed safe when administered orally, they caused no hematological changes, that differs from the data collected for the i.p., whose use leaves no doubt to be clarified by further studies at different times.

11.010 *Safety evaluation of* *Rubus rosaefolius* *extract:* *In vivo, in vitro* and *in silico* toxicological studies. Broering MF, Tonin TD, Petreanu M, Niero R, Machado ID, Santin JR Univali – Farmácia

Introduction: *Rubus rosaefolius*, popularly known as "amora-do-mato", is a medicinal plant widely used to treat pain and inflammatory disorders. However, there is lack of toxicity studies to assess potential toxicological concerns, especially, acute and sub-chronic toxicity and the possible mutagenic effects, regarding the *Rubus rosaefolius* extract. **Aim**: The aim of the study was evaluate the safety of *Rubus rosaefolius* extract using different methodologies *in vivo, in vitro and in silico.* **Methods**: The safety of *Rubus rosaefolius* extract obtained from aerial parts was assessed in acute (fixed-dose model) and subchronic toxicity (Repeated Dose 28-day Oral Toxicity) and mutagenic (micronucleous) assays in accordance with the test guidelines published by the Organization for Economic Cooperation and Development (OECD). In addition, the cytotoxicity of *Rubus rosaefolius* extract was evaluated by MTT assay in L929 cells. The hemolytic activity was performed in blood erythrocytes. The isolated compound (5,7 dihidro, 6,8,4'trimetoxi-flavonol) obtained from *Rubus rosaefolius* extract were assayed using the software T.E.S.T. (USEPA, version 4.1). All procedures were aproved by the Institutional Animal care and Use Committe (CEUA/UNIVALI 023/14). **Results**: The results obtained show that the *Rubus rosaefolius* extract when administered *in vivo* at doses of 2000 mg/kg p.o., did not cause any signs or symptoms of toxicity in mice. The same was observed in the sub-chronic toxicity assay, where did not was observed changes in the % of weight gain, water, feed consumption and the organs weight. Still, were not observed hematological or biochemical changes in animals treated with *Rubus rosaefolius* extract when compared to naive or vehicle-treated animals in both models. In addition, the extract showed no clastogenic effect on the bone marrow assay. Also the extract does not showed cytotoxic activity in L929 cells in MTT assay in all doses evaluated (0,1, 1, 10 or 100 µg/mL). In the evaluation of hemolysis *Rubus rosaefolius* extract just at the dose of 2000 mg/mL showed 50% hemolysis, the others doses do not evoke hemolysis (1, 10, 100 or 1000 µg/mL). The *in silico* toxicological prediction of the isolated compound obtained from extract showed that the compound do not exhibit mutagenic potential, but the compound could be toxic to developmental. **Conclusions**: Together, the results herein obtained indicate that *Rubus rosaefolius* extract did not presents any acute or sub-chronic toxicological effect, as well as, mutagenic or cytotoxic effect, but presents some hemolytic effect at higher dose. In this way, the *Rubus rosaefolius* extract appears to be safe as a traditional medicine for oral consumption. However, more experiments are necessary to prove the safety of 5,7 dihidro, 6,8,4'trimetoxi-flavonol compound. **Financial support information**: UNIVALI, PIBIC, CNPq (444682/2014-7)

11.011 Characterization of a cryptococcal meningitis model in male Wistar rats.
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Introduction: Cryptococcus neoformans is an opportunistic pathogen that affects commonly people with advanced HIV/AIDS and transplanted patients. This pathogen causes a severely brain infection called cryptococcal meningitis, a difficult disease to treat. To establish many treatment protocols, it is necessary to know more information about how the presence of the pathogen affects brain structure and if it influences in the penetration of the drugs. **Aims**: This study aims to develop an *in vivo* infection model for *Cryptococcus neoformans* able to be applied in studies of antifungals brain penetration. **Methods**: The experiments were approved by Animals Ethics Comitees (Protocol # 26605/2014). Based on the protocol developed by Liu et. al (2013) [1] in mice, in this model twelve male *Wistar* rats by 45 days were used. The yeast of Cryptococcus was cultured in Sabouraud dextrose 2% glucose agar for 72 hours at 37°C. The inoculum was prepared by placing three colonies into 5 mL of sterile sodium chloride solution (0,9%) and adjusted at 0,5 in MacFarland scale. Then, 100 uL of inoculum corresponding at 1.10^5 UFC/mL was injected intravenously in healthy male *Wistar* rats. On the tenth day after the establishment of the infection, the rats were euthanized and the brains were removed. Six were immediately fixed in 10% formalin and submitted to histopathological examination. The other ones were separated to determine the viable yeasts count. For that, the brains were triturated and homogenized with 5 mL of sodium chloride solution (0,9%), followed by eight serial dilutions (1: 10). Then, 50 uL of homogenized brain were cultured in Sabouraud dextrose agar plates and incubated at 37 °C and the colonies were counted 72 hours after. **Results**: All animals were infected. The average of colonies count observed after ten days by inoculation were 1,3. 10^6 UFC/g brain. In logarithmic scale it was 6,28 Log UFC/g brain. At histological analysis we could observe many agglomerated yeasts. These yeasts were round and had the presence of a prominent capsule surrounding the spherical yeast cell, with a zone of clearance or “halo” around the cells, that are characteristic of Cryptococcus. The size of the yeast cells ranges from 1-15 uM. All the areas of the brain were affected. Conclusions: This study allowed developing a model of infection within ten days. It was the first time in literature that rats were used in a model of infection to cryptococcal meningitis. In addition, these results could be used as the basis for the establishment of protocol of brain penetration. References: 1. Liu et. al, Plos Pathogens, (9), p. 1 (2013)

11.012 Local toxicity of dapaconazole, a new antifungal drug, after chronic intravaginal appplication. Campos RM, Rojas-Muscoso JA, Pissinati L, Iwamoto RD, de Nucci G Unicamp – Farmacologia

Introduction: Although imidazole antifungal drugs has been used to treat vaginal infections, the local toxicity of a new imidazole antifungal drug, dapaconazole, has not been evaluated. **Aims**: To assess local inflamation reactions in the vaginal epithelium of rabbits trated chronically with topical application of dapaconazole 2% **Methods**: Dapaconazole was singly administered by vaginal route (20mg/animal) or intravenously (0.2mg/Kg; 0.5mg/kg; 1mg/Kg) and its plasma levels were quantified by high performance liquid chromatography coupled to electrospray tanden mass spectometry. Rabbits were treated with dapaconazole 2% or placebo by daily intravaginal application (20mg/animal) for 14 days. Daily inspection of the vulva, for cutaneous reactions, was performed once before treatment period. After 14 days, all animals were killed, a macroscopic *post-mortem* examination and microscopic examination were performed. All animal protocols were aproved by the Brazilian College for Animal Experimentation (COBEA; No 2997-1). **Results** The mean area under curve of dapaconazole plasma concentration after intravaginal administration was 933ng.h/mL. A singly intravenous administration of 0.2mg/kg, 0.5mg/kg and 1mg/kg promote an area under curve of 53.82ng.h/mL, 163.582ng.h/mL and 222.0 ng.h/mL, respectively. After 14 days intravaginal application of Dapaconazole (2% cream) no mortalities or changes in body weights and food consumption was observed.. Microscopically, local signs of inflammation were seen in the vagina of placebo and dapaconazole treated groups, that consisted of minimal to moderate mixed inflammatory cell infiltrate in the epithelium and underlying connective tissue, presence of cellular debris/inflammatory cells in the lumen (which correlated with macroscopic changes) and increased thickness of epithelium. Their incidence and/or severity were slightly more pronounced in Dapaconazole treated group than in control, suggesting a mild local irritation due to the presence of the compound. **Conclusion**: A new antifungal drug was evaluated in a reliable vaginal permeability model and the vaginal histological architecture was conserved after its chronic treatment. **Sources of Research Support**: This study was supported by CNPq/Brazil

11.013 Plasma pharmacokinetics of cefazolin in obese and non-obese rats after intravenous dosing. Palma EC[1], Laureano JV[1], Lima DMF[2], Araújo BV[3], Dalla Costa T[3] – [1]UFRGS – Ciências Farmacêuticas, [2]UFRGS – Farmácia, [3]UFRGS – Farmácia

Introduction: The worldwide obesity has been increasing over the last several decades. In patients with morbid obesity [BMI ≥ 40 kg/m^2], bariatric surgery is one of the most important forms of treatment. Surgical site infections (SSIs) are one of the complications of abdominal surgery and the frequency ranges is from 1–21.7% after bariatric surgeries. To prevent SSIs cefazolin (CFZ) is the prophylactic agent of choice, although the appropriate dose for morbidly obese patients remains uncertain. At the moment, morbidly obese and non-obese patients use the same CFZ prophylactic dose (2 g, 1 h before incision). Although tissue concentrations are more relevant to antimicrobial effect, this recommendation assumes that if the pharmacokinetic/pharmacodynamic (PK/PD) plasma index obtained in obese patients is the same obtained in non-obese patients the success of the prophylaxis is warranted. **Aims**: The aim of this study was to evaluate and compare the PK of two different intravenous dosing regimens of CFZ (30 and 45 mg/kg single i.v. bolus) in obese and non-obese rats. **Methods**: All experiments were approved by Animal Ethics Committee (# 25463). Seventeen weeks old male Wistar rats obese and non-obese were used to determine the PK of CFZ in plasma. Animals were anesthetized with ethylcarbamate (1.25 g/kg i.p.) and received CFZ 30 or 45 mg/kg i.v. *bolus*. Blood samples were collected in pre-determined time intervals (0, 5, 10, 30, 45, 60, 90, 105, 120 min) up to 2 h and CFZ was assayed by LC/UV validated method. Plasma PK profiles were evaluated by non-compartmental approach and the pharmacokinetic parameters in both groups were compared by Student´s t test (= 0.05). **Results**: CFZ PK parameters after 30 mg/kg intravenous dosing in non-obese and obese rats were, respectively: elimination rate constant (k_e) of 1.11 ± 0.11 h^{-1} and 1.19 ± 0.11 h^{-1}; half-life ($t_{1/2}$) of 0.63 ± 0.07 h and 0.59 ± 0.05 h; area under the curve ($AUC_{0-\infty}$) of 198.2 ± 31.8 µg·h/mL and 187.8 ± 20,3 µg·h/mL; mean residence time (MRT) of 0.96 ± 0.03 h and 0.93 ± 0.05 h; total clearance (CL_{tot}) of 0.15 ± 0.02 L/h/kg and 0.16 ± 0.02 L/h/kg; volume of distribution (Vd) of 0.15 ± 0.02 L/kg and 0.15 ± 0.02 L/kg. After 45 mg/kg i.v. dosing in non-obese and obese rats the CFZ PK parameters were, respectively: k_e of 1.14 ± 0.08 h^{-1} and 1.11 ± 0.06 h^{-1}; $t_{1/2}$ of 0.61 ± 0.04 h and 0.63 ± 0.03 h; $AUC_{0-\infty}$ of 331.1 ± 67.5 µg·h/mL and 392.7 ± 52.2 µg·h/mL; MRT of 0.88 ± 0.10 h and 0.86 ± 0.05 h; CL_{tot} of 0.14 ± 0.03 L/h/kg and 0.12 ± 0.02 L/h/kg; Vd of 0.13 ± 0.04 L/kg and 0.10 ± 0.02 L/kg. **Conclusions**: The pharmacokinetic of CFZ showed no significant differences between non-obese and obese rats in two dosing regimens investigated (= 0.05). No dose dependency of PK parameters was observed. If plasma parameters were used, obese and non-obese individuals should receive the same dose to attain infection eradication. However, plasma PK does not show CFZ subcutaneous tissue concentrations, responsible for bactericidal effect. In this context, additional data about CFZ concentration at the target site are necessary to determine an effective prophylactic dose. **Acknowledgements**: Financial support from PPSUS/FAPERGS 2013.

11.014 Liquid chromatography/UV method for determination of cefazolin subcutaneous penetration in rats by microdialysis. Lima DMF[1], Laureano JV[2], Palma EC[2], Araújo BV[2], Dalla Costa T[2] [1]UFRGS – Farmácia, [2]UFRGS – Pharmaceutical Sciences

Introduction: Cefazolin (CFZ) is a first generation cephalosporin active against *Streptococcus*, *Staphylococcus* and *Pneumococcus*. It is largely used as antimicrobial prophylaxis in bariatric surgery and other surgical procedure in obese patients (van Kralingen et al., Eur. J. Clin. Pharmacol., 67, 985, 2011). Subcutaneous tissue penetration of CFZ at the surgical site in obese patients is unknown making it difficult to determine the adequate prophylactic dose for surgeries. Microdialysis is a sampling technique that allows for the quantification of free (pharmacologically active) antimicrobial concentrations at the infection site. No analytical method available in the literature has the adequate sensitivity to quantify the drug in microdialysate samples. **Aims**: To develop, validate evaluate the applicability of a liquid chromatography/UV (LC/UV) bioanalytical method to quantify CFZ in subcutaneous microdialysate samples of rats. **Methods**: All experiments were approved by UFRGS Animal Ethics Committee (25463). Chromatographic resolution was achieved using RP-C_{18} column (Shimadzu Shim-Pack, 250 x 4.6 mm ID; particle size 5 µm) and sodium phosphate buffer 0.01 M: acetonitrile (90: 10, *v/v*) at a flow rate of 0.8 mL/min as mobile phase. All samples were chromatographed at 30 °C, injection volume of 40 µL, with UV detection at 272 nm. Calibration curves and quality controls samples were prepared in Ringer solution. No processing of the samples was necessary prior to LC injection. For method validation, the analytical performance parameters evaluated were linearity, limit of quantification, precision and accuracy, obtained from standard curves and quality control samples, following FDA Guidance for Industry (Bioanalytical Method Validation, 2001). To demonstrate the applicability of the analytical method it was employed to quantify CFZ subcutaneous microdialysate samples obtained following 30 mg/kg i.v. *bolus* dosing to male Wistar rats (n = 10). Subcutaneous microdialysate samples were collected on anesthetized rats every 15 min for 2 h after CFZ dosing using a CMA/30 probes. CFZ microdialysate recovery was 11.6 ± 3.9%. **Results**: CFZ retention time was approximately 10 min. The method was linear for drug concentrations ranging between 25-2000 ng/mL ($r \geq 0.99$). The lower limit of quantification (LLOQ) was 25 ng/mL. The intra-day precision for quality controls ranged from 2.1% to 3.6% for day 1 and from 0.4% to 3.4% for day 2. The inter-day variation ranged from 1.0% to 5.2%. The accuracy ranged from 99.5% to 105.4%. The method was considered validated according to FDA guidelines. Following i.v. dosing, CFZ estimated subcutaneous half-life was 0.54 ± 0.08 h. The area under the free tissue curve versus time (AUC) determined by the trapezoidal rule was of 23.0 ± 2.5 µg·h/mL. The tissue penetration factor ($AUC_{tissue}/AUC_{plasma}$) was determined to be 1.2 (plasma data not shown). Conclusions: The method showed sensitivity, linearity, precision, accuracy and specificity required to quantify CFZ in subcutaneous microdialysate samples and can be used to investigate drug penetration at the surgical site in obese animals. *Acknowledgements:* Financial support from PPSUS/FAPERGS 2013.

11.015 Comparison of free subcutaneous tissue concentrations of cefazolin in obese and non-obese rats determined by microdialysis. Laureano JV[1], Palma EC[1], Lima DMF[2], Dalla Costa T[1], Araújo BV[1] – [1]UFRGS – Ciências Farmacêuticas, [2]UFRGS – Farmácia

Introduction: Cefazolin (CFZ) is used commonly to prevent surgical site infection (SSI) in patients with morbid obesity (MO) during bariatric surgery, but specific dosing guidelines are lacking. At the moment, morbidly obese and non-obese patients use the same CFZ prophylactic dose (2 g administered 1 h before incision).To be effective as prophylactic agent, CFZ concentrations reached at the surgical site have to be higher than the minimum inhibitory concentrations (MIC) of the bacterial species associated with infections in bariatric surgery. In this context, the use of a pre-clinical model of obesity can be a powerful tool to investigate CFZ tissue penetration and different dosing regimens. **Aims**: The aim of this study was to determine the *in vitro* and *in vivo* microdialysis probes recovery of and to compare CFZ penetration into subcutaneous tissue of obese and non-obese rats by microdialysis (MD) after intravenous drug dosing. **Methods**: All experiments were approved by Animal Ethics Committee (# 25463). Seventeen weeks old male obese and non-obese Wistar rats were used to determine the pharmacokinetics (PK) of CFZ in subcutaneous tissue. Animals were anesthetized with ethylcarbamate (1.25 g/kg i.p.) and the MD probe (CMA/30, 10 mm length, 6 kDa *cutoff*), inserted into the subcutaneous tissue, was perfused with Ringer's solution a constant flow rate of 4 µL/min. After 1 h for probe equilibration the animals (n =10/group) received CFZ 30 and 45 mg/kg i.v. *bolus*. Microdialysate samples were collected in pre-determined time intervals up to 2 h. MD probes were calibrated *in vitro* and *in vivo* by retrodialysis. CFZ was assayed in microdialysate samples by LC/UV validated method. Tissue PK profiles were evaluated by non-compartmental approach and the parameters in both groups were compared by Student´s t test (= 0.05). **Results**: *In vitro* CFZ probes recoveries were 12.6 ± 1.6% in average. *In vivo* recovery was 11.6 ± 3.9%. After 30 mg/kg i.v. dosing CFZ subcutaneous tissue PK parameters in non-obese and obese rats were, respectively: elimination rate constant (k_e) of 1.30 ± 0.19 h^{-1} and 1.31 ± 0.45 h^{-1}; half-life ($t_{1/2}$) of 0.54 ± 0.08 h and 0.56 ± 0.10 h; area under the curve ($AUC_{0-\infty}$) of 23.0 ± 2.5 µg·h/mL and 12.5 ± 2.8 g·h/mL; mean residence time (MRT) of 1.0 ± 0.1 h and 1.2 ± 0.1 h. After 45 mg/kg i.v. dosing CFZ subcutaneous tissue PK parameters in non-obese and obese rats were, respectively: k_e of 1.50 ± 0.44 h^{-1} and 1.23 ± 0.31 h^{-1}; $t_{1/2}$ of 0.50 ± 0.15 h and 0.59 ± 0.14 h; $AUC_{0-\infty}$ of 39.1 ± 9.3 µg·h/mL and 30.7 ± 7.1 µg·h/mL; MRT of 1.1 ± 0.2 h and 1.0 ± 0.2 h. The relation between free CFZ tissue exposure ($AUC_{tissue,free,obese}/AUC_{tissue,free,non-obese}$) after 30 and 45 mg/kg were, respectively, 0.54 and 0.78. **Conclusions**: PK parameters showed a significant difference in penetration of CFZ in the subcutaneous tissue between the obese and non-obese rats at both doses tested. The tissue concentrations of the antimicrobial decreased in obese animals and can influence the incidence of SSI.

Acknowledgements: Financial support from PPSUS/FAPERGS 2013.